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Inhibin-α, E-cadherin, calretinin and Ki-67 antigen in the immunohistochemical evaluation of canine and human testicular neoplasms

Rafal Ciaputa[1], Marcin Nowak[1], Janusz A. Madej[1], Dominik Poradowski[2], Izabela Janus[1], Piotr Dziegiel[3, 4], Elzbieta Gorzynska[5], Malgorzata Kandefer-Gola[1]

[1]Department of Pathomorphology and Forensic Veterinary Science, Chair of Pathology, Faculty of Veterinary Medicine, University of Environmental and Life Sciences, Wroclaw, Poland

[2]Department of Biochemistry, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Environmental and Life Sciences, Wroclaw, Poland

[3]Department of Histology and Embryology, Wroclaw Medical University, Wroclaw, Poland

[4]Department of Physiotherapy, University School of Physical Education, Wroclaw, Poland

[5]Histopathological Laboratory Hist-Med s.c. N.Z. M. Kosinski P. Prajs, Wroclaw, Poland

Abstract

Introduction. The steady increase of dogs with diagnosed testicular neoplasms observed in recent years prompted us to carry out immunohistochemical (IHC) studies for their better characterization. The aim of the study was to analyze most common canine testicular neoplasms (seminomas, Leydig cell and Sertoli cell tumors) with selected IHC markers and to compare the expressions of these proteins in corresponding canine and human testicular tumors.

Material and methods. Studies were carried out on testicular canine tumors: 40 cases of seminoma, 40 cases of Leydig cell tumor and 40 cases of Sertoli cell tumor. Moreover, 15 cases of human seminomas and 5 cases of human Leydig cell tumors were also analyzed. Immunohistochemistry was performed on paraffin sections by standard technique using monoclonal anti-human antibodies against E-cadherin, inhibin-α, calretinin and Ki-67. The slides were subjected to computer-aided image analysis and the intensity of the immunoreactivity was assessed by a semi-quantitative scoring system.

Results. Due to the very low prevalence of the Sertoli cell-derived tumors in the human population, we were able to examine the markers' expression only in the canine gonadal tumors. We revealed that, apart from E-cadherin in Leydig cell tumors and calretinin in seminomas, the expression of all the analyzed markers in canine and human testicular tumors was similar. *E.g.* there was no immunoexpression of inhibin-α in 75% of canine and 100% of human cases of seminoma. The immunoreactivity of Ki-67 was intense in 40% of canine and 60% of human seminomas. Immunoexpression of inhibin-α in Leydig cell tumor was intense in 70% of canine and 100% of human cases, respectively. Also the immunoreactivity of calretinin was intense in 75% of cases of canine and 100% of human Leydig cell tumors. In 50% of canine and 40% of human Leydig cell tumors, the immunoexpression of Ki-67 was weak.

Conclusions. The applied anti-human monoclonal antibodies against common antigens and markers of human testicular neoplasms could be routinely used for the immunohistochemical evaluation of canine testicular tumors.
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Key words: testicular neoplasms; dog; humans; E-cadherin; inhibin-α; calretinin; Ki-67; immunohistochemistry

Correspondence address: R. Ciaputa, DVM.
Department of Pathomorphology and Forensic Veterinary Science
Chair of Pathology, Faculty of Veterinary Medicine
Wroclaw University of Environmental and Life Sciences
C.K. Norwida St. 31, 50–375 Wroclaw
tel.: +48 71 320 54 16, e-mail: rafal.ciaputa@gmail.com

Introduction

Seminomas, Leydig cell tumors and Sertoli cell tumors represent the most frequent types of canine testicular neoplasms [1–4]. Although these tumors are also diagnosed in humans, their incidence is markedly lower than in dogs. Both Leydig cell tumors and Sertoli cell tumors are well differentiated neoplasms originating from sex cords and gonadal stroma. Seminoma is a germ cell neoplasm with relatively homogenous microscopic structure, derived from carcinoma *in situ* of germinal epithelium. According to Looijeng et al. and Bush et al., most canine seminomas show histological structure resembling that of spermatocytic seminomas diagnosed in males (humans), and the classic form of seminoma is very rarely found in dogs [5, 6]. The recently observed continuous increase in the incidence of canine testicular tumors prompted us to perform immunohistochemical (IHC) analysis of these neoplasms with such markers as E-cadherin, inhibin-α, calretinin and Ki-67, and to comparative analysis of expressions of these proteins in canine and male testicular tumors. The use of the abovementioned markers may provide detailed information on a character of testicular tumors. The expression of E-cadherin, involved in cell-cell adhesion, may provide data on a metastatic potential of a tumor, and the expression of inhibin-α, a growth inhibitor, may reflect the rate of tumor growth. This information, combined with the data on the expression of Ki-67, a cellular proliferation marker, can be helpful in establishing prognosis in dogs with testicular neoplasms. Also expression of calretinin, potential marker of secretory activity of a tumor, may be particularly useful in the case of testicular neoplasms. Furthermore, it should be emphasized that IHC analyses may frequently constitute a vital component of diagnostic process in both humans and dogs with mixed tumors or neoplasms presenting atypical histological structure. To the best of our knowledge, the expressions of markers from the abovementioned panel have not been comparatively analyzed in canine and male testicular tumors to date. Due to similarities in histological structure of male and canine testicular tumors and exposure of humans and dogs to similar environmental factors, testicular neoplasms seem to be a useful research model, enabling comparative analysis of carcinogenesis in these two species.

Material and methods

The study included specimens of canine (seminomas, Leydig cell tumors and Sertoli cell tumors, 40 each) and male testicular tumors (15 seminomas and 5 Leydig cell tumors). We did not analyze male Sertoli cell tumors, as this type of malignancy is only sporadically diagnosed in humans [7]. Canine testicular tumors were obtained during surgery or postmortem examination, from dogs of various breeds and ages. The specimens of male neoplasms were obtained from archival collection of Hist-Med s.c. N.Z. M. Kosinski P. Prajs Histopathology Unit (Wroclaw, Poland). All the diagnoses were verified histopathologically according to the respective WHO guidelines [8].

The specimens of canine testicular tumors were fixed in 7% buffered formalin (Chempur, Piekary Slaskie, Poland) for 24 h, embedded in paraffin and cut into 4 μm-thick sections. Histopathological evaluation of hematoxylin (Sigma-Aldrich, Saint Louis, MO, USA) and eosin (Chempur) stained microscopic slides was based on the WHO classification of testicular tumors [8].

The IHC analyses were conducted on 4 μm-thick paraffin sections mounted on glass slides (Dako, Glostrup, Denmark), deparaffinized in xylene (Stanlab, Lublin, Poland) and re-hydrated in alcohol gradients. The slides were heated on water bath (96°C, 20 min) with respective retrieval solutions, EnVision™ FLEX Target Retrieval Solution, High pH (Dako) for calretinin, E-cadherin and inhibin-α, or EnVision™ FLEX Target Retrieval Solution, Low pH (Dako) for Ki-67, to retrieve the antigens. The activity of endogenous peroxidase was blocked by 10 min treatment with EnVision™ FLEX Peroxidase-Blocking Reagent (Dako). Then respective primary antibodies: monoclonal mouse anti-human calretinin (clone DAK-Calret 1, 1:100, Dako), monoclonal mouse anti-human E-Cadherin (clone NCH-38, 1:50, Dako), monoclonal mouse anti-human inhibin-α (clone R1, 1:100, Dako) and monoclonal mouse anti-human Ki-67 antigen (clone MIB-1, 1:100, Dako) were applied, and the slides were incubated at room temperature for 20 min. Subsequently, the slides were washed in EnVision™ FLEX Wash Buffer (Dako), the reagents of EnVision™ FLEX/HR SM802 (Dako) visualization system were applied and slides were incubated at room temperature for 20 min. The IHC reactions were developed with 3,3-diaminobenzidine tetrahydrochloride (DAB), EnVision™ FLEX DAB+ Chromogen (Dako). Finally, the slides were rinsed in distilled water, counterstained with hematoxylin, dehydrated in alcohol gradients, passed through xylene and sealed.

The slides were subjected to computer-aided image analysis with a computer connected to Olympus BX53 optic microscope (Olympus, Tokyo, Japan) equipped with Olympus Color View IIIa digital camera (Olympus). All the measurements were carried out with the aid of cell ^ A software (Olympus).

The immunoexpression of E-cadherin, inhibin-α, and calretinin was scored according to the modified semi-quantitative IRS scale proposed by Remmele [9, 10]. The method takes into account both the proportion of positively stained cells and the intensity of the color reaction. The score constitutes the product of multiplication of both pa-

rameters and ranges from 0 to 12 points [no reaction = 0 points (–); weak reaction = 1–2 points (+), moderate reaction = 3–5 points (++), intense reaction = 6–12 points (+++)]. Expression of Ki-67 was evaluated quantitatively, based on the percentage of positively-stained cells [0–5% = no reaction (–), 6–25% = weak reaction (+), 26–50% = moderate reaction (++), above 50% = intense reaction (+++)]. Evaluation of the expression of the markers was performed by three observers with relevant experience in analyzing immunohistochemical reactions.

The results were subjected to statistical analysis with Statistica PL package (StatSoft, Krakow, Poland). The non -parametric Mann-Whitney U-test was used for intergroup comparisons of expression intensity. The threshold of statistical significance of the test was set at $p = 0.05$.

Results

Immunoexpression of the studied antigens in canine and human seminomas

The immunohistochemical analysis of testicular seminomas demonstrated clear species differences in the expression of the studied proteins. In 10% of canine testicular tumors inhibin-α expression was found to represent intense reaction, in 5% moderate reaction and in 10% weak reaction. In 75% of the studied cases no expression of the antigen could be demonstrated. In human testicular seminomas no immunoexpression of the inhibin-α antigen could be noted.

In the case of calretinin, in 50% of canine seminomas intense reaction, in 15% moderate reaction and in 5% weak reaction was detected. 30% of seminomas manifested no immunoexpression of the protein. In humans none of the seminomas studied manifested the expression.

The immunoexpression of E-cadherin in canine seminomas manifested intense reaction in 5% cases, moderate in 20% cases and weak in 25% cases. 50% of studied tumors manifested no expression of the marker. In humans intense immunoreactivity of E-cadherin in seminomas was observed in 20% tumors, moderate in 40% tumors while in 40% of male tumors no expression of E-cadherin could be demonstrated.

The immunoexpression of the Ki-67 antigen in 40% of canine seminomas represented intense, in 20% moderate and in 25% weak reaction. In 15% of tumors no expression of the Ki-67 antigen could be detected. In human seminomas intense immunoreactivity of the Ki-67 antigen was found in 60% of cases and a moderate reaction manifested in 40% (Figure 1).

Statistical analysis of the results using Mann-Whitney's test demonstrated no significant differences ($p > 0.05$) in the expressions of Ki-67, inhibin-α and E-cadhedrin and a significant difference ($p < 0.05$) in the expression of calretinin in the seminomas of dogs and men.

Immunoexpression of the studied antigens in canine and human Leydig cell tumors

Tumors of Leydig cells, similarly to seminoma, manifested expression of studied proteins at variable levels.

In canine tumors inhibin-α manifested intense reaction in 70% cases while no such expression could be detected in 30% cases. All the studied human Leydig cell tumors men showed the intense immunoreactivity of inhibin-α.

Intense reaction of calretinin was detected in 75% cases of canine Leydig cell tumors. However, 25% of examined tumors showed no expression of calretinin. In men intense reaction of calretinin was disclosed in all examined Leydig cell tumors.

In canine Leydig cell tumors intense reaction of E-cadherin was detected in 40% cases, moderate in 30% and weak in 10% cases. 20% of the canine cases manifested no expression of E-cadherin. No expression of E-cadherin was detected in the examined human tumors.

The expression of Ki-67 in canine Leydigomas was detected in 60% of cases and evaluated as a moderate reaction in 10% and in as a weak one — in 50% of the tumors; 40% of the examined tumors manifested no expression of the antigen. In human Leydig cell tumors the expression of Ki-67 was weak and detected in 40% of cases while 60% of the tumors manifested no expression of the Ki-67 antigen (Figure 2).

Statistical analysis of the results using Mann-Whitney's test demonstrated no significant differences ($p > 0.05$) in the expressions of Ki-67, calretinin and inhibin-α between canine and human Leydig cell tumors, however, a significant difference between both species was found in the expression of E-cadherin.

Immunoexpression of the studied antigens in canine Sertoli cell tumors

Neoplastic lesions originating from the supporting Sertoli cells were subjected to immunohistochemical analysis only in canine testes. In 30% of the tumors immunoreactivity of inhibin-α was evaluated as intense, in 25% of them as moderate and in 20% as weak reaction. In 25% of the cases no expression of the protein was detected.

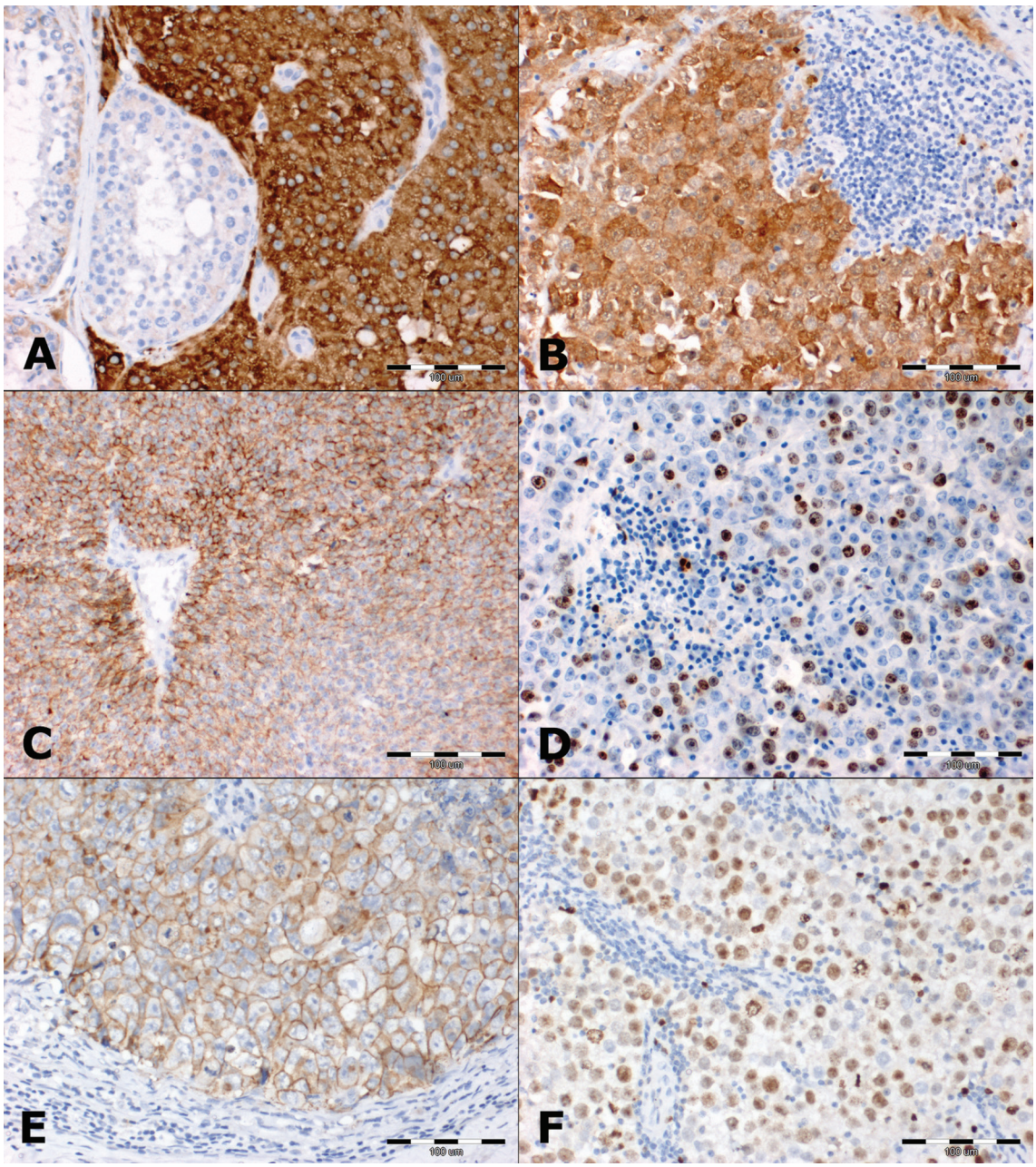

Figure 1. Presence of the studied markers in dog seminoma: (A–D — dog; E, F — man). **A.** Cytoplasmic expression of inhibin-α (× 400); **B.** Cytoplasmic and nuclear (in individual cells) expressions of calretinin (× 400); **C.** Weak cytoplasmic expression of E-cadherin and strong membrane expression of this protein (× 400); **D.** Nuclear expression of Ki-67 antigen (× 400); **E.** Expression of E-cadherin in seminoma (× 400); **F.** Nuclear expression of Ki-67 in seminoma cells (× 400)

The immunoreactivity of calretinin in 25% of cases of Sertoli cell tumors was intense, in 15% — moderate and in 10% — weak. In the entire material 50% of the tumors manifested no expression of calretinin.

In the case of E-cadherin moderate reaction was demonstrated in 20% of cases and the weak one in 15% cases. In 65% cases no expression of the antigen was detected.

The immunoreactivity of proliferative antigen Ki-67 was intense in 20% of Sertoli cell tumors, in 15% — moderate, and in 20% — weak. In 45% of the cases no expression of the protein was detected (Figure 3).

To compare the immunoreactivity of all studied antigens in both human and canine testicular tumors the data were summarized up and presented in a graph form in Figure 4.

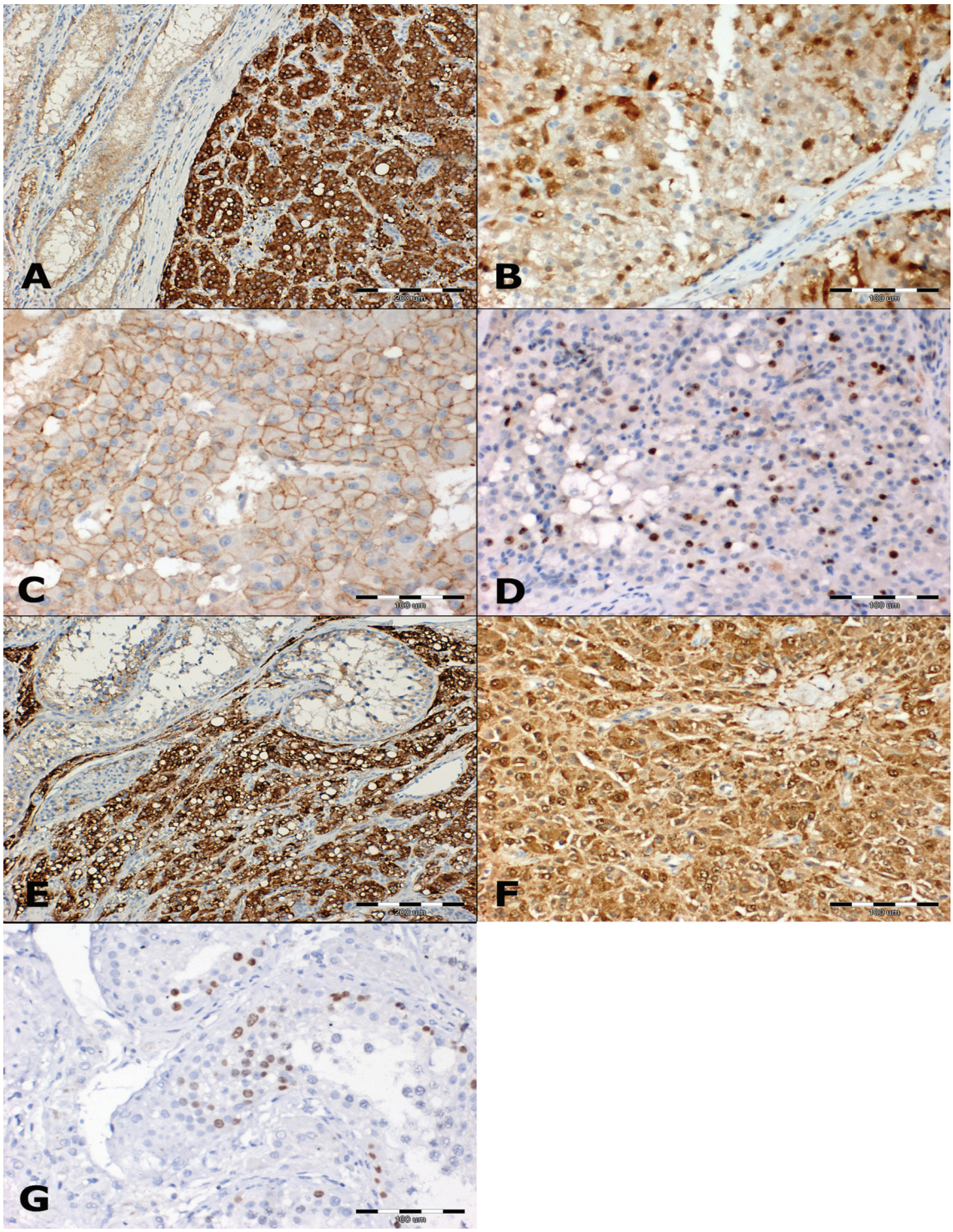

Figure 2. Presence of the studied markers in Leydig cell tumor: (A–D — dog; E–G — man). **A.** Strong cytoplasmic expression of inhibin-α (× 200); **B.** Cytoplasmic and nuclear expression of calretinin (× 400); **C.** Membranous expression of E-cadherin (× 400); **D.** Nuclear expression of Ki-67 (× 400); **E.** Inhibin-α in the cytoplasm of Leydig cell tumor (× 200); **F.** Strong cytoplasmic and nuclear expression of calretinin (× 400); **G.** Nuclear expression of Ki-67 (× 400)

Discussion

Our study was prompted by constant increase in the incidence of canine testicular tumors and resultant growing number of cases whose appropriate identification raises some controversies and requires additional IHC evaluation [4, 11, 12]. Moreover, we searched for biological characteristics which would be

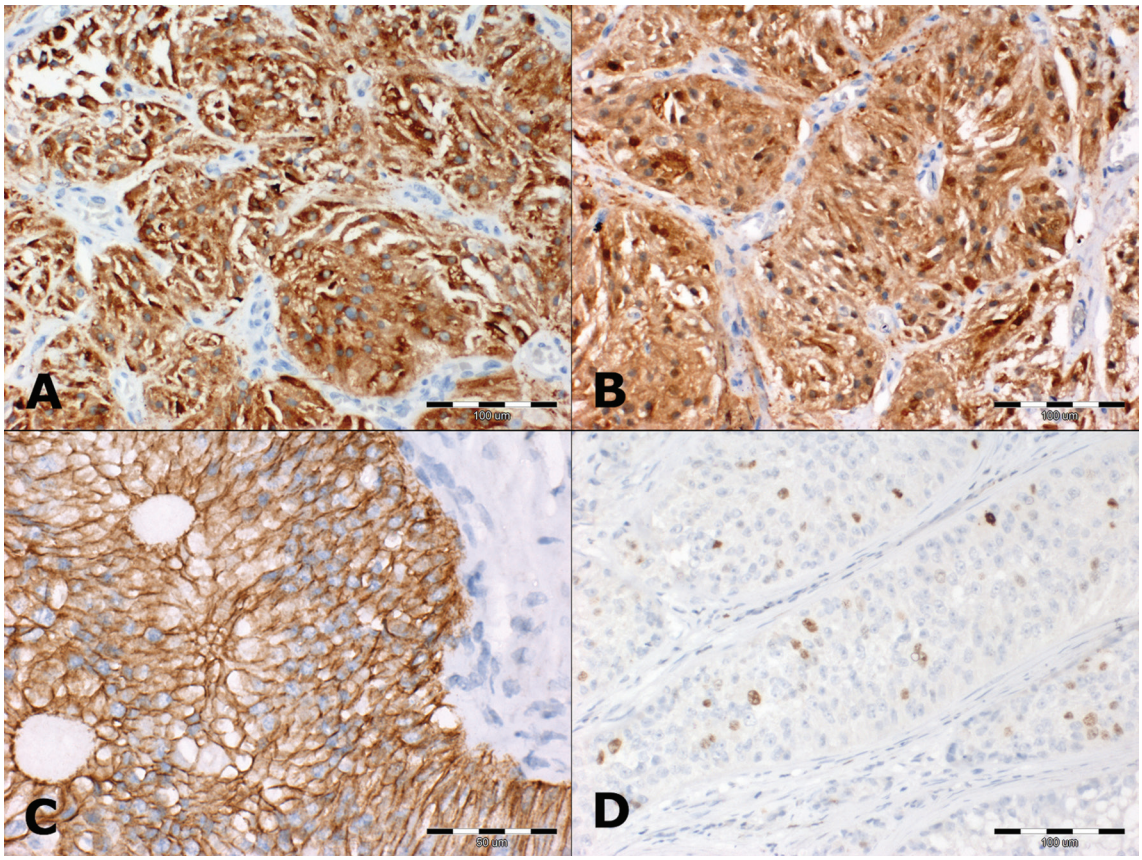

Figure 3. Presence of the studied markers in canine Sertoli cell tumors. **A.** Expression of inhibin-α in the cytoplasm (× 400); **B.** Cytoplasmic and nuclear expression of calretinin (× 400); **C.** Membrane expression of E-cadherin (× 600); **D.** Nuclear expression of Ki-67 (× 400)

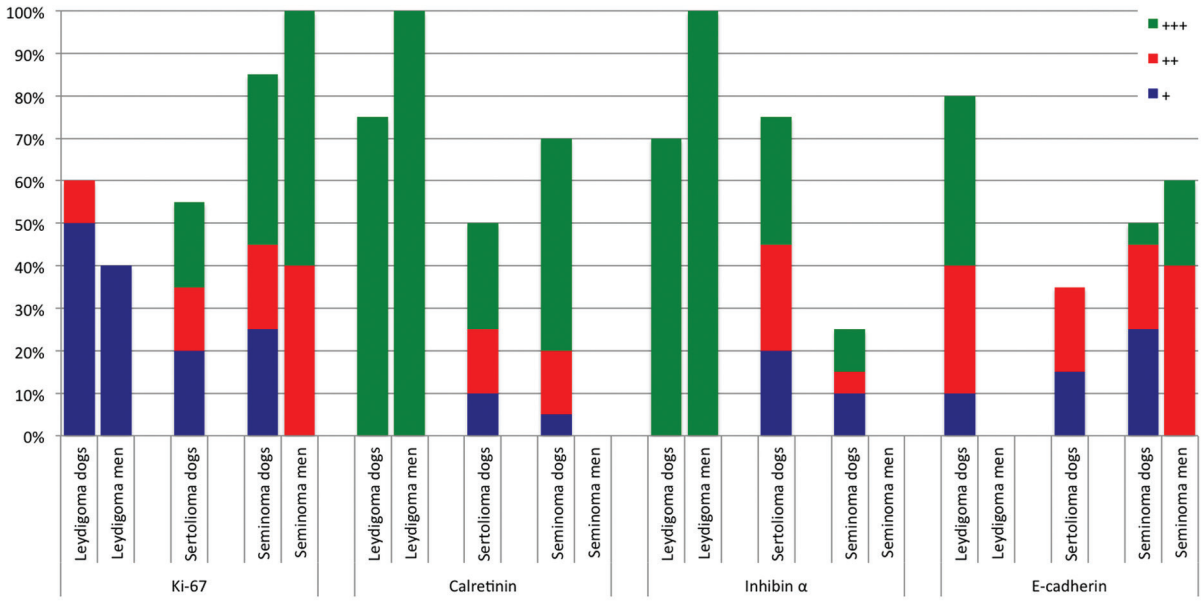

Figure 4. The summary of the assessment of immunoreactivity of all analyzed markers in canine and male testicular tumors. Y axis: the percentage of cells with the described intensity of immunoreactivity which was measured semi-quantitatively as described in Methods

helpful in the optimization of the therapy of canine testicular tumors.

We analyzed the expression of a growth inhibitor, inhibin-α [13], as a marker of tumor growth potential. Inhibin-α is a polypeptide gonadal hormone, for the first time isolated from follicular fluid of cattle and pigs in 1985 [14–16]. It is responsible for the selective inhibition of FSH secretion [17]. Inhibin-α is composed of two subunits, α and β; the latter one comprises two subunits, A and B [18]. As inhibin-α is synthesized by Sertoli and Leydig cells, these two cellular types show its strong cytoplasmic expression on IHC examination [14, 19–22]. We documented strong cytoplasmic expression of inhibin-α in canine testicular neoplasms, too; it was the strongest in Leydig cell tumors and somehow weaker in Sertoli cell tumors. This observation is consistent with previous reports on the expression of this protein in canine [17, 21] and male testicular tumors [23]. The fact that we observed the cytoplasmic expression of inhibin-α also in a small fraction of canine seminomas, may point to some differences in the biology of the latter tumor type in dogs and humans.

Calretinin is a neuronal calcium-binding protein which belongs to the calmodulin superfamily and acts as an intracellular regulator of Ca^{2+} concentration [24–26]. The extraneuronal expression of calretinin can be observed in both normal and neoplastic epithelial cells of the ovaries, uterus, testes, thymus and adrenal glands [27]. Previous studies documented both cytoplasmic and nuclear expression of this protein; however, the exact mechanism and role of the extraneuronal expression of calretinin are still not fully understood [27]. As strong expression of calretinin was observed in cells involved in the synthesis of steroid hormones, this protein was postulated to be a marker of secretory activity of ovarian or testicular tumors [27, 28]. In our study, the immunoreactivity of calretinin was the strongest in the case of canine and male Leydig cell tumors, *i.e.* in neoplasms with potential hormonal activity. The canine and human specimens did not differ significantly in terms of the calretinin expression intensity. Also Radi and Miller [27] documented expression of calretinin in all types of canine testicular tumors; in contrast, Leydig cell tumors are the only testicular neoplasms showing expression of this protein in humans [29].

E-cadherin, a protein involved in cell-cell adhesion, and thus associated with metastatic potential of neoplastic tumors [30, 31] is a transmembrane calcium-dependent protein of the cadherin family, together with often studied N- and P-cadherins [32, 33]. E-cadherins form complexes on the cytoplasmic side of the plasma membrane with another group of proteins, catenins. Impaired formation of the cadherin-catenin complexes is reflected by decreased intercellular adhesion and results in increased invasive and metastatic potential of a tumor [32–36]. We observed strong membrane expression and weaker cytoplasmic expression of E-cadherin in canine testicular tumors. Most of the canine and male neoplasms analyzed in our study showed similar intensity of E-cadherin expression. The only exception pertained to Leydig cell tumors, in which the expression of E-cadherin was stronger, corresponding to 6–12 points of the Remmele scale. It has been assumed that lower expression of E-cadherin is associated with greater mobility of cancer cells, which is reflected by the increased risk of tumor spread [32].

The nuclear expression of Ki-67 antigen serves as a basis for mitotic index determination, thus being a marker of tumor cells' proliferation [37–39]. Ki-67 belongs to the group of non-histone nuclear proteins [40, 41]. The expression of this protein can be observed already during G_1 phase of the cell cycle; it increases markedly during S and G_2 phases, reaches its peak level during M phase, and is absent in G_0 cells. Consequently, Ki-67 is detected mostly in proliferating cells [41, 42]. Determination of the mitotic index with an aid of anti-Ki-67 antibody constitutes an indirect method for measuring tumor aggressiveness. Both canine and male seminomas showed the strongest expression of Ki-67 in all testicular tumors analyzed in our study, however, with only slightly weaker immunoreactivity in Leydig cell tumors. Also Papaioannou et al. showed that the nuclear expression of Ki-67 is the strongest in seminomas, and somehow weaker in other types of canine testicular tumors [43]. The usefulness of Ki-67 antigen as a marker of cell proliferation was also confirmed in human testicular germ cell tumors [44].

Our study revealed that, apart from E-cadherin in Leydig cell tumors, the immunoreactivity of all the analyzed markers in canine and male testicular tumors was similar. This suggests that the antibodies tested in our study could be routinely used in IHC evaluation of canine testicular tumors. The appropriate interpretation of the expression of hereby examined proteins may be helpful in the selection of optimized treatment and establishing a prognosis in dogs with testicular neoplasms.

Moreover, the hereby documented analogies in the expression patterns of the selected markers/ /antigens suggest that canine tumors may present a useful model for the studies of carcinogenesis in human testes.

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References

1. Ciaputa R, Nowak M, Kiełbowicz M, Antończyk A, Błasiak K, Madej JA. Seminoma, sertolioma, and leydigoma in dogs: clinical and morphological correlations. *Bull Vet Inst Pulawy.* 2012;56:361–367.
2. Maiolino P, Restucci B, Paparella S, Paciello O, De Vico G. Correlation of nuclear morphometric features with Animal and Human World Health Organization International histological classification of canine spontaneous seminomas. *Vet Pathol.* 2004;41:608–611.
3. Meuten DJ. Tumors in domestic animals. In: MacLachlan JM, Kennedy PC. 4th ed. *Tumors of the Genital Systems.* Iowa State Press: Iowa; 2002:561–573.
4. Nowak M, Madej JA, Ciaputa R, Poradowski D. Manifestation of tumours in domestic animals in Lower Silesia in 2005–2008. *Bull Vet Inst Pulawy.* 2010;54:229–236.
5. Looijenga LH, Olie RA, Van der Gaag I et al. Seminomas of the canine testis. Counterpart of spermatocytic seminoma of men? *Lab Invest.* 1994;71:490–496.
6. Bush JM, Gardiner DW, Palmer JS, Rajpert-De Meyts E, Veeramachaneni DN. Testicular germ cell tumours in dogs are predominantly of spermatocytic seminoma type and are frequently associated with somatic cell tumours. *Int J Androl.* 2011;34:288–295.
7. Zhou M, Magi-Galluzzi C. Genitourinary Pathology: A Volume in Foundations in Diagnostic Pathology Series. In: Levin HS, ed. *Neoplasms of the Testis.* Elsevier: Philadelphia; 2007:582–588.
8. Kennedy PC, Cullen JM, Edwards JF et al. *Histological Classification of Tumors of the Genital System of Domestic Animals.* Armed Forces Institute of Pathology; Washington 1998.
9. Remmele W, Stegner HE. Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue. *Pathologe.* 1987;8:138–140.
10. Brouckaert O, Paridaens R, Floris G, Rakha E, Osborne K, Neven P. A critical review why assessment of steroid hormone receptors in breast cancer should be quantitative. *Ann Oncol*. 2013;24:47–53.
11. Ciaputa R, Kandefer-Gola M, Nowak M, Madej JA. Prevalence of tumours in domestic animals in the Lower Silesia (Poland) in 2009–2011. *Bull Vet Inst Pulawy.* 2013;57:53–59.
12. Grieco V, Riccardi E, Greppi GF, Teruzzi F, Iermano V, Finazzi M. Canine testicular tumours: a study on 232 dogs. *J Comp Path.* 2008;138:86–89.
13. Zheng W, Lauchlan SC. Inhibin and activin: their roles in ovarian tumorigenesis and their diagnostic utility in surgical pathology practice. *Applied Immunol Mol Morph.* 1999;7:29–38.
14. Kommoss F, Oliva E, Bittinger F et al. Inhibin-alpha CD99, HEA125, PLAP, and chromogranin immunoreactivity in testicular neoplasms and the androgen insensitivity syndrome. *Hum Pathol.* 2000;31:1055–1061.
15. Ling N, Ying SY, Ueno N et al. Isolation and partial characterization of a Mr 32,000 protein with inhibin activity from porcine follicular fluid. *Proc Natl Acad Sci.* 1982;82:7217–7221.
16. Robertson DM, Foulds LM, Leversha L et al. Isolation of inhibin from bovine follicular fluid. *Biochem Biophys Res Commun.* 1985;126:220–226.
17. Taniyama H, Hirayama K, Nakada K et al. Immunohistochemical detection of inhibin-α, -βB, and -βA chains and 3β-hydroxysteroid dehydrogenase in canine testicular tumors and normal testes. *Vet Pathol.* 2001;38:661–666.
18. Matias-Guiu X, Pons C, Prat J. Müllerian inhibiting substance, alpha-inhibin, and CD99 expression in sex cord-stromal tumors and endometrioid ovarian carcinomas resembling sex cord-stromal tumors. *Hum Pathol.* 1998;29:840–845.
19. Henley JD, Young RH, Ulbright TM. Malignant Sertoli cell tumors of the testis: a study of 13 examples of a neoplasm frequently misinterpreted as seminoma. *Am J Surg Pathol.* 2002;26:541–550.
20. McClugagge WG, Shanks JH, Whiteside C, Maxwell P, Banerjee SS, Biggart JD. Immunohistochemical study of testicular sex cord-stromal tumors, including staining with anti-inhibin antibody. *Am J Surg Pathol.* 1998;22:615–619.
21. Owston MA, Ramos-Vara JA. Histologic and immunohistochemical characterization of a testicular mixed germ cell sex cord-stromal tumor and a Leydig cell tumor in a dog. *Vet Pathol.* 2007;44:936–943.
22. Reis-Filho JS, Ricardo S, Gartner F, Schmitt FC. Bilateral gonadoblastomas in a dog with mixed gonadal dysgenesis. *J Comp Pathol.* 2004;130:229–233.
23. Nista M, Pastrián LG, González-Peramato P, De Miguel MP. Inhibin bodies: a new marker for immature Sertoli cells. *Histopathology.* 2011;58:1019–1027.
24. Cao QJ, Jones JG, Li M. Expression of calretinin in human ovary, testis, and ovarian sex cord-stromal tumors. *Int J Gynecol Pathol.* 2001;20:346–352.
25. Katsuaki S, Yoshimichi U, Aya S et al. Large cell calcifying Sertoli cell tumor of the testis: Comparative immunohistochemical study with Leydig cell tumor. *Pathol International.* 2005;55:366–371.
26. Lugli A, Forster Y, Haas P et al. Calretinin expression in human normal and neoplastic tissues: A tissue microarray analysis on 5233 tissue samples. *Hum Pathol.* 2003;34:994–1000.
27. Radi ZA, Miller DL. Immunohistochemical expression of calretinin in canine testicular tumours and normal canine testicular tissue. *Res Vet Sci.* 2005;79:125–129.
28. Bertschy S, Genton CY, Gotzos V. Selective immunocytochemical localisation of calretinin in the human ovary. *Histochem Cell Biol.* 1998;109:59–66.
29. Augusto D, Leteurtre E, De La Taille A, Gosselin B, Leroy X. Calretinin: a valuable marker of normal and neoplastic Leydig cells of the testis. *Appl Immunohistochem Mol Morphol.* 2002;10:159–162.
30. Heimann R, Lan F, McBride R, Hellman S. Separating favorable from unfavorable prognostic markers in breast cancer: the role of E-cadherin. *Canc Res*. 2000;60:298–304.
31. Silye R, Karayiannakis AJ, Syrigos KN et al. E-cadherin/ /catenin complex in benign and malignant melanocytic lesions. *J Pathol.* 1998;186:350–355.
32. Nowak M, Madej JA, Dzięgiel P. Expression of E-cadherin, β-catenin and Ki-67 antigen and their reciprocal relationships in mammary adenocarcinomas in bitches. *Folia Histochem Cytobiol.* 2007;45:233–238.
33. Nozawa N, Hashimoto S, Nakashima Y et al. Immunohistochemical alpha- and beta-catenin and E-cadherin expression and their clinicopathological significance in human lung adenocarcinoma. *Pathol Res Pract.* 2006;202:639–650.
34. Miao Y, Li A, Wang L et al. Expression of p130cas, E-cadherin and β-catenin and their correlation with clinicopathological

parameters in non-small cell lung cancer: p130cas over-expression predicts poor prognosis. *Folia Histochem Cytobiol.* 2012;50:392–397.
35. Asgeirsson KS, Jonasson JG, Tryggvadottir L et al. Altered expression of E-cadherin in breast cancer: patterns, mechanisms and clinical significance. *Eur J Cancer.* 2000;36:1098–1106.
36. Epstein RJ. Human molecular biology: an introduction to the molecular basis of health and disease. In: *Adhesion molecules and the extracellular matrix*. 1st ed. Cambridge University Press: Cambridge; 2002:209–234.
37. Enami Y, Kato H, Murakami M et al. Anti-transforming growth factor-β1 antibody transiently enhances DNA synthesis during liver regeneration after partial hepatectomy in rats. *J Hepatobiliary Pancreat Surg.* 2001;8:250–258.
38. Niiya T, Murakami M, Aoki T, Murai N, Shimizu Y, Kusano M. Immediate increase of portal pressure, reflecting sinusoidal shear stress, induced liver regeneration after partial hepatectomy. *J Hepatobiliary Pancreat Surg.* 1999;6:275–280.
39. Bieniek A, Pula B, Piotrowska A et al. Expression of metallothionein I/II and Ki-67 antigen in various histological types of basal cell carcinoma. *Folia Histochem Cytobiol.* 2012;50:352–357.
40. Gerdes J, Shwab U, Lemke H, Baisch H, Wacker HH. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated witch cell proliferation. *Int J Canc.* 1983;31:13–20.
41. Gerdes J, Li L, Schlueter C et al. Immunobiochemical and molecular biologic characterization of the cell proliferation-associated nuclear antigen that is defined by monoclonal antibody Ki-67. *Am J Pathol*. 1991;138:867–873.
42. Brown DC, Gatter KC. Monoclonal antibody Ki-67: its use in histopathology. *Histopathology.* 1990;17:489–503.
43. Papaioannou N, Psalla D, Zavlaris M, Loukopoulos P, Tziris N, Vlemmas I. Immunohistochemical expression of dog TERT in canine testicular tumours in relation to PCNA, ki67 and p53expression. *Vet Res Commun.* 2009;33:905–919.
44. Kayaselçuk F, Polat A, Kilinç F, Güvel S, Tuncer İ. p53, Fas (APO 1) and Ki-67 immunostaining in testicular germcell tumors and correlation with pathologic stage, tumor size, necrosis, and vascular invasion. *Turk J Cancer.* 2003;33:137–143.

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